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Complete Genome Sequence of *Clostridium perfringens* LLY_N11, a Necrotic Enteritis-Inducing Strain Isolated from a Healthy Chicken Intestine

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ABSTRACT *Clostridium perfringens* strain LLY_N11, a commensal bacterium, which previously induced necrotic enteritis in an experimental study, was isolated from the intestine of a young healthy chicken. Here, we present the complete genome sequence of this strain, which may provide a better understanding of the molecular mechanisms involved in necrotic enteritis pathogenesis.

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Clostridium perfringens is a Gram-positive, spore-forming, and anaerobic bacterium responsible for a variety of diseases, such as gas gangrene, bacteremia, and food poisoning in humans and necrotic enteritis (NE) in food animals (1–4). Commensal *C. perfringens* strains are widely distributed in nature, especially in the soil and in the intestines of humans and animals (1, 5). Annual economic loss caused by NE is estimated to be over $6 billion globally in the poultry industry (6). In our previous report, strain LLY_N11, isolated from the intestine of a healthy chicken, was found to belong to *C. perfringens* and induced NE in an experimental model (7). In this study, the whole-genome sequencing of *C. perfringens* strain LLY_N11 was conducted to characterize potential virulence factors involved in molecular pathogenesis.

A sample was prepared for genome sequencing by culturing strain CP15 anaerobically overnight at 37°C in brain heart infusion nutrient broth (Becton, Dickinson and Company, Sparks, MD, USA). The genomic DNA was then extracted from the cultures using a cetyltrimethylammonium bromide method (8). The complete genome sequence of *C. perfringens* LLY_N11 was determined with the PacBio RS II platform (Pacific Biosciences, Menlo Park, CA, USA) at the Institute for Genomic Sciences, University of Maryland at Baltimore (Baltimore, MD, USA). The *de novo*-assembled whole-genome shotgun sequence was verified with the Illumina HiSeq 4000 platform (Illumina, Inc., San Diego, CA, USA) by Novogene, Inc. (Sacramento, CA, USA). Gene prediction and functional analysis were carried out using EDGE bioinformatics tools (9) and the NCBI Prokaryotic Genome Annotation Pipeline (http://www.ncbi.nlm.nih.gov/genome/annotation_prok). Multiple-genome alignment showed that strain LLY_N11 has an average nucleotide identity value of 99.1% to *C. perfringens* reference strain ATCC 13124 and a 99.9% identity score to *C. perfringens* strains Del1 and JP55 (7).

The complete genome sequence of strain LLY_N11 contained 3,346,739 bp in one chromosome with a G+C content of 28.5%, 3,031 coding sequences (total), 30 rRNAs, 90 tRNAs, and 4 noncoding RNAs. Three plasmids, designated pLLY_N11_1, pLLY_N11_2, and pLLY_N11_3, comprised 13,363 bp, 14,754 bp, and 72,060 bp, respectively. The genome of strain LLY_N11 was analyzed for the presence of antibiotic resistance genes

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(10) and virulence factors (Virulence Factor Database, http://www.mgc.ac.cn/VFs). *C. perfringens* strain LLY_N11 possessed *mepA*, *tet38*, *tetA*(P), and *tetB*(P), all of which are associated with tetracycline resistance; it also contained the antibiotic resistance gene *mprF* and the rifampin resistance gene *rpoB*. Computational analysis revealed that strain LLY_N11 contained 20 toxin genes, including alpha-toxin, alpha-clostripain, enterotoxins (EntA, EntB, and EntD), hemolysin, kappa-toxin, mu-toxin, and beta2-toxin. Alpha-toxin (encoded by *cpa*) was the most toxic extracellular enzyme that hydrolyzed important constituents of eukaryotic cell membranes (11, 12), while beta2-toxin was found to be associated with intestinal disorders in chickens and other food animals (13, 14). Based on the toxin gene types (1), *C. perfringens* strain LLY_N11 is a new strain of *C. perfringens* type A due to absence of beta, iota, and epsilon toxin.

Accession number(s). This whole-genome sequence has been deposited at GenBank under the accession numbers CP023410 (chromosome), CP023411 (plasmid pLLY_N11_1), CP023412 (plasmid pLLY_N11_2), and CP023413 (plasmid pLLY_N11_3).

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The PacBio sequencing was completed using the genomic platform at the Institute for Genome Sciences, University of Maryland at Baltimore (Baltimore, MD, USA). The Illumina sequencing was performed by Novogene, Inc. (Sacramento, CA, USA). We also thank Ransom Baldwin for critically reading the manuscript.

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